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Preventive effect of *Aegle marmelos* leaf extract on isoprenaline-induced myocardial infarction in rats: biochemical evidence

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Abstract

We have evaluated the preventive effects of an aqueous *Aegle marmelos* leaf extract (AMLEt) in isoprenaline (isoproterenol)-induced myocardial infarction in rats. Rats were pretreated with AMLEt (50, 100 or 200 mg kg^{-1}) for 35 days. After the treatment period, isoprenaline (200 mg kg^{-1}) was administered subcutaneously to rats at an interval of 24 h for two days. The activity of creatine kinase (CK) and lactate dehydrogenase (LDH) was significantly increased in serum and significantly decreased in heart of isoprenaline-treated rats. Pretreatment with AMLEt decreased the activity of CK and LDH in serum and increased them in the heart. The activity of sodium–potassium dependent adenosine triphosphatase (Na^+K^+ATPase) was significantly decreased while the activity of calcium dependent adenosine triphosphatase (Ca^{2+}ATPase) was simultaneously increased in the heart and aorta. AMLEt pretreatment increased the activity of Na^+K^+ATPase and decreased the activity of Ca^{2+}ATPase in the heart and aorta simultaneously. The levels of cholesterol and triglycerides increased, while the levels of phospholipids decreased in the heart and aorta of isoprenaline-treated rats. In AMLEt-pretreated rats the levels of cholesterol and triglycerides decreased whereas phospholipids increased in heart and aorta. All the deranged biochemical parameters were restored with 200 mg kg^{-1} AMLEt. Similarly α-tocopherol (60 mg kg^{-1})-pretreatment to isoprenaline-treated rats exhibited a significant effect on all the parameters studied. The results from this study may have clinical relevance.

Introduction

Myocardial ischaemia occurs when myocardial oxygen demand exceeds oxygen supply and as a result it causes in-cell injury known as myocardial infarction, which is one of the most lethal manifestations of cardiovascular disease (Mohanty et al 2004). The generation of toxic, reactive oxygen species such as superoxide radical, hydrogen peroxide and hydroxyl radical leads to the damage of myocardial cells (Vaage & Valen 1993). Isoprenaline (isoproterenol), a synthetic catecholamine, has cardiotoxic effects on the myocardium. Auto-oxidation of catecholamines releases cytotoxic free radicals. Generation of free radicals is induced by isoprenaline and these free radicals can lead to cardiac damage (Nirmala & Puvanakrishnan 1994). Use of plants and herbs for the treatment of cardiovascular diseases in Ayurveda and other indigenous systems of medicine has given a new lead to understanding the pathophysiology of these diseases.

Aegle marmelos (Linn.) Correa Roxb (Rutaceae) is distributed widely in India, Pakistan, Myanmar and Bangladesh. The stem, bark, root, leaves and fruits of *A. marmelos* exhibit a variety of medicinal properties. In Ayurvedic medicine, the leaves of *A. marmelos* are mostly used in the treatment of diarrhoea, dysentery, palpitation of heart and eye diseases (Kirtikar & Basu 1993). Haravey (1968) reported that the leaves of *A. marmelos* possessed a 'cardiotonic' effect on frog heart. We have already reported the antioxidant and antilipoperoxidative properties of aqueous *A. marmelos* leaf extract in isoprenaline-treated Wistar rats (Rajadurai & Stanely Mainzen Prince 2005; Rajadurai et al 2005). This study reports the efficacy of aqueous *A. marmelos* leaf extract (AMLEt) on tissue lipids and membrane bound enzymes in

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isoprenaline-induced myocardial infarction. We have compared the preventive effect of AMLEt and α-tocopherol in isoprenaline-treated Wistar rats.

Materials and Methods

Plant extract

Aqueous *Aegle marmelos* leaf extract (brown dry powder) was received as a gift from Chemiloids, Vijayawada, Andhra Pradesh, India. It was botanically authenticated by Dr V. Venkatasalu, Botanist, Department of Botany, Annamalai University, Annamalainagar, India. Herb to product ratio was 6:1. The extract was suspended in distilled water before use (Rajadurai & Stanely Mainzen Prince 2005).

Chemicals

Isoprenaline hydrochloride (isoproterenol hydrochloride) and digitonin were purchased from Sigma Chemical Company (St Louis, MO). All other biochemicals and chemicals used in the study were of analytical grade.

Animals

All the experiments were carried out in female albino Wistar rats (150–170 g), obtained from the Central Animal House, Rajah Muthiah Institute of Health Sciences, Department of Experimental Medicine, Annamalai University, Tamil Nadu, India. They were housed in polypropylene cages ($47 \times 34 \times 20$ cm) lined with husk, which was renewed every 24 h, under a 12:12 h light/dark cycle at approximately 22°C. The animals had free access to tap water and a standard pellet diet (Pranav Agro Industries Ltd, Maharashtra, India). The pellet diet consisted of 21% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose and 55% nitrogen free extract (carbohydrates). The diet provided metabolizable energy of 3000 kcal kg^{-1}. This study was approved by the Ethical Committee of Annamalai University (Approval No. 108; Dated 21.10.2002).

Induction of myocardial infarction

Rats were pretreated with AMLEt for 35 days and after the treatment period, isoprenaline (isoproterenol) hydrochloride (200 mg kg^{-1}) dissolved in normal saline was administered subcutaneously at an interval of 24 h for two days (Rajadurai & Stanely Mainzen Prince 2005).

Experimental design

A total of 42 rats were used in the experiment. The rats were divided into seven groups of six rats each. Group 1, control rats; group 2, normal rats orally treated with aqueous AMLEt (200 mg kg^{-1}) for 35 days; group 3, rats subcutaneously injected with isoprenaline (200 mg kg^{-1}, dissolved in saline, once a day for two days). The rats in groups 4, 5 and 6 were orally pretreated with aqueous AMLEt (50, 100 or 200 mg kg^{-1}, respectively) for 35 days and then subcutaneously injected with isoprenaline (200 mg kg^{-1} once a day for two days) (Rajadurai & Stanely Mainzen Prince 2005). The rats in group 7 were orally pretreated with α-tocopherol (60 mg kg^{-1}) for 35 days and then subcutaneously injected with isoprenaline (200 mg kg^{-1}, once a day for two days) (Rajadurai & Stanely Mainzen Prince 2005).

After the last treatment, all the rats were killed by cervical decapitation after an overnight fast. Blood was collected and serum separated. Heart and aorta were excised immediately, and rinsed in ice-chilled normal saline. A known weight of the tissue was homogenized in appropriate buffer solution. The homogenate was centrifuged at low speed for 5 min. The supernatant was used for the estimation of various biochemical parameters.

Assay of CK, LDH, ATPases and estimation of lipids

The activity of creatine kinase (CK) and lactate dehydrogenase (LDH) was assayed by the methods of Okinaka et al (1961) and King (1965), respectively. Activity of Na^+K^+ATPase and Ca^{2+}ATPase was assayed by the methods of Bonting (1970) and Hjerton & Pan (1983), respectively. Liberated phosphorus was estimated by the Fiske & Subbarow method (Fiske & Subbarow 1925). Lipids were extracted by the method of Folch et al (1957). The total, ester and free cholesterol were estimated by the methods of Zlatkis et al (1953) and Varley et al (1991), respectively. Triglycerides were estimated by the method of Foster & Dunn (1973) and phospholipid content was estimated by the method of Zilversmit & Davis (1950).

Statistical analysis

Statistical analysis was performed using SPSS Software package, version 6.0. The values were analysed by one way analysis of variance followed by Duncan's multiple range test (Duncan 1957). All the results were expressed as mean ± s.d. for six rats in each group. $P < 0.05$ was considered as significant.

Results and Discussion

Table 1 shows the activity of CK and LDH in serum and heart of normal and experimental rats. The activity of these enzymes was significantly increased in serum and significantly decreased in heart of isoprenaline-treated rats. Pretreatment with AMLEt at doses of 100 or 200 mg kg^{-1} exerted a significant effect on the activity of CK and LDH in isoprenaline-treated rats.

Figure 1 shows the heart weight of normal and experimental rats. Heart weight was increased significantly in isoprenaline-administered rats. Oral pretreatment with AMLEt (100 or 200 mg kg^{-1}) for a period of 35 days showed a significant decrease of heart weight in isoprenaline-treated rats.

Table 1 Effect of *Aegle marmelos* leaf extract on the activity of creatine kinase (CK) and lactate dehydrogenase (LDH) in serum and heart of normal and isoprenaline-treated rats

Group	Serum CK ($IU\,L^{-1}$)	Heart CK (μmol phosphorus liberated min^{-1} $(mg\ protein)^{-1}$)	Serum LDH ($IU\,L^{-1}$)	Heart LDH (nmol pyruvate liberated min^{-1} $(mg\ protein)^{-1}$)
Group 1	231.4 ± 6.8^{a}	12.4 ± 1.0^{a}	72.61 ± 3.8^{a}	102.5 ± 6.4^{a}
Group 2	220.6 ± 8.0^{a}	12.4 ± 1.9^{a}	71.8 ± 4.1^{a}	102.3 ± 4.7^{a}
Group 3	384.0 ± 16.2^{b}	6.9 ± 0.8^{b}	133.2 ± 8.6^{b}	74.1 ± 5.0^{b}
Group 4	362.1 ± 20.5^{bc}	7.7 ± 0.6^{bc}	116.0 ± 15.8^{c}	78.6 ± 6.1^{bc}
Group 5	301.7 ± 11.5^{c}	8.4 ± 0.8^{c}	109.7 ± 4.4^{c}	83.5 ± 4.6^{c}
Group 6	270.4 ± 10.6^{d}	10.2 ± 0.9^{d}	88.5 ± 5.1^{d}	88.2 ± 5.1^{ad}
Group 7	274.3 ± 12.1^{d}	10.9 ± 1.0^{d}	92.0 ± 3.7^{d}	90.7 ± 4.8^{d}

Each value is mean ± s.d. for six rats in each group. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (Duncan's multiple range test).

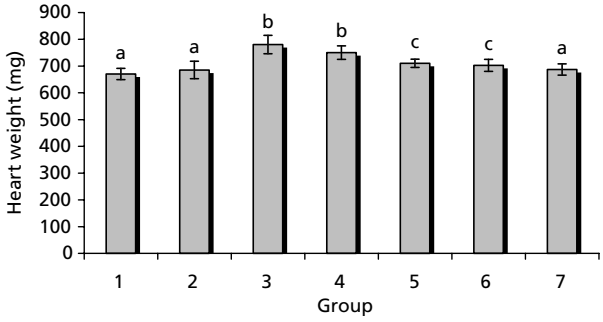

Figure 1 Effect of AMLEt on heart weight in normal and isoprenaline-treated rats. Each value is mean ± s.d. for six rats in each group. Values not sharing a common superscript (a, b, c) differ significantly at $P < 0.05$ (Duncan's multiple range test). Group 1, control rats. Group 2, normal rats + AMLEt ($200\,mg\,kg^{-1}$). Group 3, isoprenaline-treated rats. Group 4, AMLEt-treated rats ($50\,mg\,kg^{-1}$) + isoprenaline. Group 5, AMLEt-treated rats ($100\,mg\,kg^{-1}$) + isoprenaline. Group 6, AMLEt-treated rats ($200\,mg\,kg^{-1}$) + isoprenaline. Group 7, α-tocopherol ($60\,mg\,kg^{-1}$)-treated rats + isoprenaline.

Table 2 Effect of *Aegle marmelos* leaf extract on the activity of $Na^{+}K^{+}$ATPase and Ca^{2+}ATPase in heart and aorta of normal and isoprenaline-treated rats

Group	$Na^{+}K^{+}$ATPase (μmol phosphorus liberated h^{-1} $(mg\ protein)^{-1}$)		Ca^{2+}ATPase (μmol phosphorus liberated h^{-1} $(mg\ protein)^{-1}$)	
	Heart	Aorta	Heart	Aorta
Group 1	3.31 ± 0.23^{a}	2.25 ± 0.10^{a}	1.81 ± 0.13^{a}	2.06 ± 0.12^{a}
Group 2	3.32 ± 0.08^{a}	2.28 ± 0.08^{a}	1.82 ± 0.12^{a}	2.06 ± 0.08^{a}
Group 3	2.25 ± 0.08^{b}	1.29 ± 0.08^{b}	3.47 ± 0.21^{b}	3.46 ± 0.23^{b}
Group 4	2.36 ± 0.13^{b}	1.33 ± 0.13^{b}	3.38 ± 0.30^{b}	3.34 ± 0.07^{b}
Group 5	2.77 ± 0.12^{c}	1.43 ± 0.08^{bc}	3.06 ± 0.13^{c}	2.91 ± 0.11^{c}
Group 6	2.89 ± 0.12^{d}	1.65 ± 0.12^{d}	2.36 ± 0.23^{d}	2.60 ± 0.19^{d}
Group 7	2.85 ± 0.14^{d}	1.71 ± 0.14^{d}	2.47 ± 0.25^{d}	2.53 ± 0.20^{d}

Each value is mean ± s.d. for six rats in each group. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (Duncan's multiple range test).

The activity of membrane-bound enzymes such as $Na^{+}K^{+}$ATPase and Ca^{2+}ATPase in heart and aorta are shown in Table 2. Isoprenaline-administered rats showed a significant decrease in the activity of $Na^{+}K^{+}$ATPase and a significant increase in the activity of Ca^{2+}ATPase in heart and aorta. AMLEt (100 or $200\,mg\,kg^{-1}$) pretreatment to isoprenaline-treated rats for a period of 35 days increased the activity of $Na^{+}K^{+}$ATPase and decreased the activity of Ca^{2+}ATPase significantly in heart and aorta.

The concentration of total, ester and free cholesterol in heart and total cholesterol in aorta of normal and isoprenaline-administered rats is shown in Table 3. Isoprenaline-administered rats showed a significant increase in total cholesterol in heart and aorta. Free and ester cholesterol was also increased in the heart of isoprenaline-treated rats. There was a significant increase in triglyceride level in the heart and aorta. A significant decrease was observed in the myocardial phospholipids but there was no significant difference in aorta of isoprenaline-treated rats (Table 3). Oral pretreatment with AMLEt (100 or $200\,mg\,kg^{-1}$) for 35 days exerted a significant effect on tissue cholesterol, phospholipids and triglycerides in isoprenaline-treated rats.

For all the parameters studied, pretreatment with AMLEt at doses of 100 or $200\,mg\,kg^{-1}$ showed significant effects when compared with isoprenaline-treated rats. AMLEt at a dose of $50\,mg\,kg^{-1}$ did not show any significant effect. AMLEt ($200\,mg\,kg^{-1}$) treatment to normal rats did not show any significant effect on any of the biochemical

Table 3 Effect of *Aegle marmelos* leaf extract on the concentration of lipids in heart and aorta in normal and isoprenaline-treated rats

Group	Heart (mg (g wet tissue)$^{-1}$)					Aorta (mg (g wet tissue)$^{-1}$)		
	Total cholesterol	Free cholesterol	Ester cholesterol	Triglycerides	Phospholipids	Total cholesterol	Triglycerides	Phospholipids
Group 1	5.23 ± 0.41^{a}	2.13 ± 0.18^{a}	3.25 ± 0.22^{a}	3.53 ± 0.26^{a}	24.1 ± 0.80^{a}	3.58 ± 0.18^{a}	2.28 ± 0.21^{a}	18.3 ± 1.35^{a}
Group 2	5.02 ± 0.33^{a}	2.02 ± 0.13^{a}	3.18 ± 0.20^{a}	3.49 ± 0.21^{a}	24.8 ± 1.33^{a}	3.50 ± 0.16^{a}	2.16 ± 0.18^{a}	18.5 ± 0.83^{a}
Group 3	8.74 ± 0.62^{b}	3.75 ± 0.23^{b}	5.85 ± 0.36^{b}	4.41 ± 0.23^{b}	13.0 ± 0.92^{b}	5.32 ± 0.22^{b}	3.14 ± 0.23^{b}	16.4 ± 1.36^{a}
Group 4	8.51 ± 0.40^{b}	3.52 ± 0.21^{b}	5.06 ± 0.40^{b}	4.12 ± 0.40^{b}	14.2 ± 1.42^{b}	5.20 ± 0.19^{bc}	3.02 ± 0.30^{bc}	16.6 ± 1.56^{a}
Group 5	7.93 ± 0.36^{c}	3.18 ± 0.26^{c}	4.23 ± 0.33^{c}	3.91 ± 0.32^{c}	16.1 ± 1.41^{c}	5.02 ± 0.18^{bc}	2.98 ± 0.26^{bc}	16.7 ± 1.26^{a}
Group 6	6.63 ± 0.32^{d}	2.78 ± 0.20^{d}	3.73 ± 0.26^{d}	3.82 ± 0.26^{d}	18.5 ± 0.74^{d}	4.56 ± 0.24^{d}	2.75 ± 0.25^{c}	17.2 ± 1.65^{a}
Group 7	6.50 ± 0.30^{d}	2.64 ± 0.23^{d}	3.56 ± 0.28^{d}	3.78 ± 0.22^{d}	19.2 ± 1.38^{d}	4.51 ± 0.27^{d}	2.78 ± 0.23^{c}	17.3 ± 1.53^{a}

Each value is the mean ± s.d. for six rats in each group. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT).

parameters studied. α-Tocopherol pretreatment showed a significant effect on all the parameters studied. The effect exerted by AMLEt 200 mg kg^{-1} was found to be similar to the effect exerted by α-tocopherol (60 mg kg^{-1}).

Isoprenaline-administration in rats leads to increased peroxidation of lipids and eventually extensive necrosis of the cell membrane. As a result of necrosis, selected marker enzymes of serum CK and LDH increase in myocardial infarction (Paritha et al 1996). The increased activity of these enzymes in serum with subsequent decrease in heart might be due to their leakage from the heart as a result of isoprenaline-induced necrosis. Similar results have been reported in isoprenaline-treated rats (Paritha et al 1996). AMLEt pretreatment to isoprenaline-treated rats for 35 days declined the activity of these enzymes in serum and increased in the heart.

An increase in the wet weight of the heart of isoprenaline-administered rats was observed. This could have been due to the increased water content, oedematous intramuscular space and extensive necrosis of cardiac muscle fibres followed by invasion of the damaged tissues by inflammatory cells (Nirmala & Puvanakrishnan 1994). Pretreatment with AMLEt decreased heart weight in isoprenaline-treated rats.

Membrane bound enzymes such as Na^+K^+ATPase and Ca^{2+}ATPase play a significant role in maintaining normal ion levels within the myocytes (Vajreswari & Narayanareddy 1992). Any alteration in the properties of these ion pumps affects the function of heart. There is a report showing that the failure of the cell membrane to maintain normal transmembrane ionic distribution through ion pumps is considered to be a major event in the pathogenesis of ischaemia and arrhythmias (Vajreswari & Narayanareddy 1992).

A decrease in Na^+K^+ATPase activity and an increase in Ca^{2+}ATPase activity was observed in isoprenaline-treated heart and aorta. Na^+K^+ATPase is a lipid dependent enzyme containing the 'SH' group. The decreased Na^+K^+ATPase activity might have been due to elevated lipid peroxidation by free radicals in isoprenaline-treated rats (Paritha et al 1996). Increased activity of Ca^{2+}ATPase could have been due to the activation of adenylate cyclase by isoprenaline (Paritha et al 1996). Similar findings have been reported in isoprenaline-treated rats (Paritha et al 1996). AMLEt pretreatment increased the activity of Na^+K^+ATPase and decreased the activity of Ca^{2+}ATPase in isoprenaline-treated rats.

We observed high levels of cholesterol in isoprenaline-treated rats. An increased level of cholesterol increases the risk of myocardial infarction. The increased concentration of cholesterol in tissues could be due to a decrease in HDL cholesterol since HDL cholesterol is known to be involved in the transport of cholesterol from the tissues to the liver for its catabolism (Mathew et al 1981). Alterations in lipid composition observed in isoprenaline-treated cardiac tissue might have been due to the destruction of cardiomyocytes (Borinski et al 1993). Paritha & Devi (1997) reported an increase in the lipid levels in isoprenaline-treated rats and this might have been due to enhanced lipid biosynthesis by cardiac cAMP.

In our study, we observed an increase in triglycerides and a decrease in phospholipids in heart and aorta in isoprenaline-treated rats. The observed increase in triglycerides might have been due to a decrease in the activity of lipoprotein lipase, resulting in decreased uptake of triglycerides from the circulation. Cell membranes are a rich source of phospholipids and degradation of phospholipids results in membrane dysfunction, which leads to cell injury. These altered levels of phospholipids might have been due to the enhanced membrane degradation (Sushmakumari et al 1990). Pretreatment with AMLEt to isoprenaline-treated rats for a period of 35 days showed a preventive effect in all the lipid parameters studied. α-Tocopherol treatment also showed a preventive effect on the lipid profile studied in isoprenaline-treated rats.

Phytochemical studies reveal the presence of various constituents such as alkaloids, flavonoids and sterols. The chemical constituents of *Aegle marmelos* leaves are listed in Table 4 (Indian Council of Medical Research 1987). Previous studies have shown that plant sterols, flavonoids and alkaloids possess lipid-lowering effects (Kremmer et al 1979; Koshy et al 2001; Vanstonea et al 2001). The lipid-lowering effect of AMLEt might have been due to the presence of these chemical constituents in the leaves.

Table 4 Chemical constituents of A*egle marmelos* leaves (Indian Council of Medical Research 1987)

Aegelin
Aegelinine
Cineole
Citrol
Citronellal
Cuminaldehyde
D-limonene
Fagarine
Gamma-sitosterol
N-2 methoxy-2(4-methoxy phenyl)-ethylcinnamide
N-2 methoxy-2-(4-methoxy phenyl)-ethylcinnamamide
N-2 methoxy-2-(4{3′,3′-dimethyl allyloxy}-ethylcinnamide
O-(3,3-dimethylallyl)-halfordinol
P-cymene
Phellandrene
Skimmianine

Conclusion

Oral pretreatment of aqueous AMLEt had an antihyperlipidaemic effect in isoprenaline-induced myocardial infarction rats. The antioxidant phytochemicals present in the AMLEt scavenged free radicals and indirectly helped to decrease lipids by reducing or inhibiting the lipid peroxidation process. However, further studies are necessary to find out the exact mechanism of action of the lipid lowering property of AMLEt. The results from this study may have clinical relevance.

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